

EDITORIAL ARTICLES.

ON THE PRESENT STATE OF KNOWLEDGE IN BACTERIAL SCIENCE IN ITS SURGICAL RELATIONS.

(Continued from Page 60.)

STERILIZATION—CULTURE SOILS.

In order to work with perfectly pure apparatus, instruments, etc., it is necessary that they should all be perfectly sterilized beforehand, that is, they should all be made free from germs which adhere to all articles that have been exposed to the air. This sterilization is the chief feature of the whole of bacterial science, and not only, as an art, requires a very great amount of practice to secure perfection in it, but necessitates a certain kind of mental training and intellectual bias in order at all times to fully realize the whole extent of damage which a slight inaccuracy or omission in conducting the experiments may lead to.

This sterilization must apply not only to the vessels used and the soils contained in them, but to all instruments used in transferring the germs, and to the surfaces which have to be penetrated in order to procure the matter for inoculation, and to the hands of the operators as well, in all manipulations of the latter kind. Recent investigators¹ have proved that the instruments and hands of the operators are by far a more frequent source of undesired infection, than the particles suspended in the air.

According to the different objects to be sterilized, different methods are, therefore, put into practice. And it must be borne in mind, that as we have to deal with different phases of development of the micro-organisms—(as the bacteria themselves, and their spores, from which the bacteria are supposed to develop in the course of their evolution)—the process which will destroy the one will not always destroy the

¹ Herman Kümmell, Die Contact und Luftinfection in der Chirurgie. Deutsche med. Wochenschrift. 1885. No. 22.

others as well—the spores being the more resistant both to chemical agents and to alterations of temperature.

The most reliable method of sterilization is by heat; this is applicable to all vessels, instruments, etc., and suffices, if properly applied, to destroy all the bacteria and spores which should by chance adhere to the apparatus.

Before sterilizing, the vessels, etc., must be thoroughly cleansed, which is done with the help of mineral acids, water, distilled water, alcohol and ether successively and in this order; the alcohol absorbing the last drops of the water and the ether, in like manner, the alcohol. The ether is allowed to evaporate. The necks of the vessels are then well plugged with cotton wads, which are generally sterilized at the same time with the vessels. The cotton admits of a circulation of air within the vessel, but filters off the germs suspended in the atmosphere. Although the cotton prevents any bacteria or spores from falling on the surface of the soils enclosed in the vessels, yet under certain circumstances fungi falling on top of the cotton may develop and, forming a mycelium, may grow through the whole substance of the cotton and, appearing at the under side of the plug, be brushed down into the vessel, when the plug is removed. When, therefore, a vessel plugged with cotton is to remain a long time exposed to the air, it is advisable to protect the upper surface of the cotton with some impermeable tissue.

The vessels thus prepared are then placed inside of a specially constructed metal box—"hot box"—composed of sheet-iron, with double walls enclosing an air-space. This apparatus is heated directly over a gas-flame, a thermometer registering the inside temperature and another instrument called the thermo-regulator graduating the heat by directly controlling the gas-supply by means of a column of mercury, which rises or sinks through expansion by the heat, and thus narrows or widens the passage-way admitting the gas.

The hot-box is kept at a temperature of 150° C. for a period of two hours. Apparatus thus sterilized is generally kept in the hot-box till used.

Metal instruments, such as knives, scissors, forceps, etc., may be

sterilized by heating them directly in a Bunsen flame; they are then layed on sterilized glass plates and protected by covers.

For sterilizing rubber objects, liquids, and certain kinds of culture-soils, which could not withstand so great a degree of dry heat, an apparatus is used called the *steaming apparatus* of Koch, Gaffky and Löffler. It consists of a large cylinder about half a metre in height and twenty centimetres in diameter, or more, manufactured of strong tin and surrounded by a coat of thick felt or asbestos to prevent the radiation of heat. A conical lid, holding a thermometer, sets loosely upon it, and at the juncture of the middle and lower third an iron grating is transversely fixed inside. Upon this grating the objects to be sterilized are placed, and, the space underneath being filled with water, a number of Bunsen burners are placed under the cylinder and the water heated to boiling. As soon as this is accomplished the cylinder is filled with steam, and *the thermometer registers 100° C.* This condition is kept up for a period varying from half an hour to two hours, according to the size of the articles to be sterilized. This apparatus is preferred to the "digestor," formerly much in use to sterilize fluids with hot steam under an increased atmospherical pressure, as being much simpler and equally effective. Immersion in boiling oil, paraffine, etc., which will often suffice for sterilization, is also less convenient; while simple boiling of a fluid is not sufficient to sterilize it (Schroeder¹). (Pasteur, of milk²). But some fluids, like blood-serum, cannot be heated to the boiling point of water without becoming coagulated; and yet it is desirable to sterilize them. Now a temperature above 52° C. will destroy all the bacteria and will not effect the coagulation of albuminous media. The spores, however, not being damaged by such degrees of heat, in the course of a few days, develop into bacteria; and these bacteria may then be killed by a repeated moderate heating (Tyndall). The apparatus in use for such purposes consists of a cylinder made of copper with double walls and furnished with a hollow cover. The space between the walls, and the cover is filled with water; the water is then warmed by gas flames, one being placed under the cylinder, the other under a lateral protuber-

¹ Annalen der Chemie und Pharmacie, 1861. Vol. 117, p. 273.

² Compt. rend., 1860. Vol. 50, p. 849.

ance of the cover. Three thermometers indicate the temperatures of the interior air-space and the two water-compartments respectively. The whole is covered with felt, and furnished with water gauges, escape-pipes, etc.

In this apparatus the charged vessels are kept at a temperature of 58° C. for a period of one or two hours for five or eight days successively. Other objects which cannot be exposed to heat at all, as the hands of the operators, etc., are sterilized by means of one-in-thousand aqueous solution of corrosive sublimate; the cleansing having been effected with soap and water and ether.

Turning to the preparation of cultivating media after Koch's methods, we must give our attention to potatoes, gelatine, isinglass and blood-serum soils.

The potatoes are cleansed by means of a nail brush and water, without injuring their epidermis; they are then laid in a one to five promille solution of corrosive sublimate for a period of about one hour, in order to sterilize them. The sublimate must then be again washed off with water, in order not to prevent the desired growth of the culture. The potatoes are then boiled for about one hour in the steaming apparatus in a suitable vessel and are then placed in covered dishes which are lined with filtering paper well dampened with corrosive sublimate solution. When cool they are cut in halves with sterilized cold knives, a fresh knife being employed for each potato, and the left hand of the operator, holding the potato, being freshly sterilized. The bacteria are then inoculated on the cut surfaces by means of a sterilized platinum wire, and the whole left in the moist glass chamber to develop under cover. The colony then, if the atmosphere be kept moist, soon begins to grow in every direction, and soon assumes a characteristic appearance resembling lichens on stones, and may be easily distinguished by its location from any accidental cultures having developed from germs which may have fallen on the soil from out of the atmosphere at the time when the cover was lifted off for the purpose of inoculating. If no germ be inoculated the potato pieces will remain perfectly sweet for an indefinite period, in the moist chamber.

Gelatine soils are prepared in the following manner: One pound of

finely cut or minced lean meat is placed in a dish, one liter of distilled water poured upon it, and the whole left in an ice chest for twenty-four hours. The infusion is then strained through gauze and enough distilled water added to make one liter. Ten gm. of dry peptone, 5 gm. common salt and 50 gm. of purest gelatine, cut into small pieces, are then added. When the gelatine has deliquesced in the course of an hour or more, it may be thoroughly dissolved by the application of moderate heat. The liquid is then usually acid in its reaction and must be rendered neutral or slightly alkaline by the addition of a sufficient quantity of carbonate of soda or carbonate of potassa. The mixture is then warmed up till it comes to a boil, and must then be filtered while still warm. To effect this a funnel is used made of copper with double sides, and lined with glass. The space between the walls is filled with warm water; the water is kept warm by a gas-flame applied to a projecting part of the funnel. The filtered liquid is then poured into sterilized test tubes or other vessels, and the sterilized cotton wads are replaced in the openings. These glasses are then sterilized by discontinuous heating as before described. The test-tubes are then ready for use. Instead of using the foregoing compound (Loeffler) as a soil, any other suitable soil may be used. Thus Hueppe gives the following receipt: Peptone 3%, sugar $\frac{1}{2}\%$, extract of beef $\frac{1}{2}\%$, gelatine 5%. In fact any of the former fluid media of Pasteur, etc., may be rendered solid by addition of a sufficient quantity of gelatine.

The liquefaction of the gelatine is often undesirable in cases where a growth is to be observed at a higher temperature than that at which gelatine remains solid. In such cases soils prepared with agar-agar, or Japanese isinglass, may be used. One and one-half or 2 per cent solutions of this substance are used. After the fine cuttings have been soaked in water for twenty-four hours in an ice-chest, they are thoroughly dissolved by boiling; the hot solution is then neutralized with carbonate of soda, and then boiled for two hours continuously in the steaming apparatus. The fluid is then to be filtered, either through gauze or through cotton or glass-wool inside of the straining apparatus (Rosenbach). Otherwise the technique does not differ from that of the gelatine.

In other cases blood-serum can be used as a soil for germ-cultures, since it can be rendered at once solid and transparent.

Sheep or ox-blood may be used, and it should be received at once into sterilized glass-stoppered vessels, the skin of the animal where the puncture is made having first been thoroughly cleansed and rendered aseptic and the first drops of blood having been allowed to escape. The vessels are then put away in an ice chest.

After about thirty hours the transparent serum may be drawn off by means of a sterilized pipette and filled into sterilized test tubes, which are then recovered with cotton and sterilized by discontinuous heating in the copper apparatus above described.

After perfect sterilization the serum is to be rendered solid, which is done in an apparatus consisting of a tin box with double sides and bottom, furnished with a glass top and covered with a layer of felt. The walls contain water, and heat is directly applied to the bottom. The blood-serum coagulates at 85° C., and becomes opaque; at a temperature of 65° C., however, it becomes solid, but remains transparent. The most perfect results are achieved if the temperature be kept between 65° and 68° C. The box is so placed that the floor occupies a slanting position, and so that the test tubes lie in an inclined and nearly horizontal position, the cotton wad designating the highest point; by this means the serum is solidified in a thinner layer and presents a larger surface for inoculation. Infusion of meat to an amount of 33%, peptone and grape sugar to an amount of 1%, common salt to $\frac{1}{2}\%$, and carbonate of soda to neutralize the solution, may be added to the blood-serum if desired. These ingredients should be previously mixed together and sterilized, and added to the serum after cooling. The whole must then be sterilized by the discontinuous method.

Some species of microbes grow on blood-serum soils and on none of the other ones mentioned. This fact is especially noticeable in some pathogenic germs.

Thus far we have only spoken of filling vessels and test tubes with the soils. But great advantages are sometimes to be gained by growing cultures on plane surfaces. For this purpose microscopic *slides* are used, having been previously duly sterilized. A small amount of liquefied gelatine is taken from one of the vessels with a sterilized

pipette and allowed to run out on the slide into a convenient layer for observation. They are then kept between sterilized glass dishes in a moist atmosphere, as was described in regard to the potato-cultures, and may be arranged in stacks by means of small sterilized glass ferules and plates.

The main advantage of these slide-cultures is the readiness with which they can be observed under the microscope, with low powers. If high powers are to be used it is only necessary to cover one of the colonies to be observed with a cover-glass, and then even immersion systems can be made use of.

It is advisable to use a more adherent soil for these slide-cultures and 10% gelatine soil is generally used in preference to the 5% solution used in the test tubes. A 2% agar-agar soil corresponds to a 10% gelatine.

As a rule these slide cultures cannot be kept pure for a great length of time, in consequence of germs from the air and apparatus falling upon the surface of the soil. But this is a matter of secondary importance, since the germs invading from without always develop at the point where they fall, and can be distinguished with the eye or with a lens from the inoculated colonies, as well as by their localization.

The *inoculations* of soils are performed with the greatest possible caution. A piece of platinum wire melted into a long glass rod forms the *inoculating needle*, which may be very quickly sterilized by heating it to a red heat in the flame of a spirit lamp; it is then allowed to cool each time before using.

The cotton wad is then loosened in the mouth of the test tube, where it has generally become impacted by the sterilization process, by means of forceps sterilized in the flame. The test tube is then turned upside down and held in an inverted position, while the inoculation needle is charged by dipping it into the matter to be examined. The cotton is then quickly removed by other fingers of the same hand holding the needle, and the needle thrust a considerable distance up into the gelatine. In this way the inoculation puncture is obtained, some of the material being deposited on the surface of the gelatine at the point where the needle entered, and some being distributed along the course of the puncture in the gelatine. The colonies developing

in these two different localizations differ in so far as the air of the atmosphere has access to the one and not to the other. The needle is then withdrawn, the cotton replaced and the test tube put aside in a convenient stand for observation.

Care must be taken in handling test tubes with gelatine soils, not to inclose them in the palm of the hand; the gelatine becomes liquified at blood heat, and the cultures can no longer remain strictly localized in a liquid soil.

If the cotton have been exposed to the air it is serviceable to burn the upper part in a flame before removing the wad, so as to destroy any germs that may have developed there.

Inoculations into agar-agar soils are conducted in the same manner. Blood-serum is only used for surface cultivations, it being not sufficiently transparent in thick layers. The inoculating needle, charged with the germs, is simply drawn over the oblique surface of the solidified serum, the test tube being held horizontally.

Slide soils are inoculated in a similar manner. The platinum wire charged with the matter to be examined is drawn quickly across the gelatine surface in parallel lines at right angles to the length of the slide. This is best done just after the gelatine has been spread upon the slide while it is cooling, when it is just at the point of solidifying. In this manner the colony is inclosed in the gelatine lying just beneath the surface.

The matter to be inoculated should also receive attention, so as to save the trouble of uselessly inoculating other germs than those intended. In inoculating matter from dead subjects the transferring of the secretions or parts of organs should be accomplished as soon as possible after death. The skin to be cut through should first be cleansed and disinfected with corrosive sublimate solution (1 in 1,000) and kept moist during the manipulations, to prevent the germs rising into the air. The first incisions through the skin are then performed with recently sterilized or hot instruments (Koch). All succeeding incisions are performed with a new set of hot instruments, fresh forceps, scissors, knives, etc., being employed for each successive layer of tissue—the material to be inoculated, however, must not be touched but with cold sterilized instruments, for fear of destroying

the vitality of the germs to be examined. In this manner the danger of contaminating the material used for inoculation with micro-organisms from the peripheral portions of the organ is avoided. Excised organs are first to be washed in a one in twenty solution of carbolic acid for the space of ten minutes and subsequently for five minutes, in a one-pro-mille solution of corrosive sublimate for the purpose of destroying the spores, before cutting them open and proceeding to inoculate as above described (Loeffler).

If syringes are to be employed they must be constructed without India-rubber, so that they may be perfectly sterilized by heat. The glass piston is wound about with cotton and silk thread each time anew and the whole sterilized at a temperature of 150° C. before using. The air-tight fitting is secured by means of fresh pieces of cork inserted between the glass cylinder and the metal mountings. Similar precautions are necessary when inoculating animals with the different germs.

As we have seen, the main object is to attain pure cultures, that is to say, colonies formed only of one single species of germ. But since the matter to be examined most usually contains a mixture of different kinds of micro-organisms it is necessary to separate them. This object is achieved by means of plate cultures. Large pieces of plate-glass, 10 by 12 centimetres, having been duly cleaned and sterilized, 10% gelatine soil is liquified by heat in its vessel, and while in a liquid state, is inoculated with a trace of the matter by means of the sterilized needle. The vessel is then shaken so as to distribute the germs throughout the fluid, and when the gelatine begins to solidify it is poured out upon the glass plate. These glass plates are then stacked in moistened glass covered dishes and put by for observation.

By this means the germs are separated, and can develop into *colonies* at different points in the gelatine; they can be studied with the magnifying glass, and can be subsequently separately inoculated into other plate cultures, onto slides, and from there into test tubes, etc. In this manner pure cultures can be comparatively easily obtained.

If the number of germs contained in the matter to be examined be too great, a drop of the matter may be first added to a quantity of sterilized distilled water and a trace of this water, after agitating, be used to inoculate the fluid gelatine. Or, series of test tubes with fluid

gelatine may be used to raise the first inoculation to a second, third or fourth dilution, by transferring one or more drops from one tube into the next, and so on, and then preparing plate cultures.

If, however, the germs to be investigated will only grow on blood-serum, special care must be taken to have them in as pure a state as possible from the start.

The cultures all present a characteristic appearance to the naked eye, and especially when viewed with a lens. They differ in color, transparency, humidity, gloss, etc. They often affect the soil, changing its color, its consistency, its odor. Thus many micro-organisms liquefy the gelatine soils when they develop, and some more rapidly than others; in the one case the gelatine is completely turned to a liquid in its upper portion; in the other, only a funnel-shaped zone of liquid is formed. Some species of organisms can only grow on the surface of gelatine soils, where the air has access, and are impeded in their development when a thin piece of isinglass is laid over the colonies upon the surface of the gelatine. Others will only develop when the soils (agar-agar) are kept at a certain temperature, as that of the body. Again the development of germs differs with the soil and with its constitution, its acidity, the presence of grape or cane-sugar, peptone, etc.

The methods detailed may appear in part very circumstantial and tedious, but there is hardly any science which requires so strict an adherence to details and where a desire to expedite matters and to shorten the methods is followed by such disastrous results.

SUPPURATION IN GENERAL.

Blood of the healthy subject was formerly very generally believed to contain micro-organisms; and even when Pasteur, Burdon Sanderson and Klebs denied this, many observers still held to their former opinion till Koch was enabled by his improved methods of staining and illuminating to corroborate the statements of the above-named authorities.¹ But pus from phlegmonous suppurations had always been known to contain micrococci, and, therefore, as soon as it was proved that micro-organisms did not *normally* exist in the tissues it ap-

¹ R. Koch. Untersuchungen über die Aetiologie der Wundinfections Krankheiten. Leipzig. 1878. Translated by W. Watson Cheyne, Sydenham Soc. 8o.

peared natural to suppose that all acute suppurations were produced by the presence of micro-organisms, and accordingly we find this to have been the prevailing opinion of pathologists during the last ten or twenty years, based upon certain writings of such authors as Hueter, Klebs, Lister, Kocher, Koch and Ogston.

At this time Uskoff, working with the assistance of Ponfick, of Breslau, published a series of experiments on dogs,¹ in some of which he had succeeded in obtaining suppuration without, as he believed, introducing bacteria into the tissues. He found that chemically indifferent fluids, as milk, distilled water, olive oil, when introduced into the subcutaneous cellular tissue only once, and in small quantities, did not excite suppuration, but that larger doses or repeatedly injected doses did excite active inflammation and suppuration. Even pus containing micro-organisms did not always call forth suppuration when small quantities were employed. Some of the inflammatory products contained organisms, others did not. Chemically irritating liquids, as croton oil, oil of turpentine, produced suppuration in all cases except when minute quantities were injected with olive oil, and in all but one of the severe inflammatory processes obtained by this method micro-organisms were absent.

Uskoff concluded that suppuration did not always depend upon the presence of micro-organisms. Although it was subsequently clearly proved that many of Uskoff's experiments were not convincing, that his technique was faulty, his methods of sterilization inadequate, and, therefore, his conclusions not reliable, yet his paper was of very considerable importance, both on account of the incitement it gave to further experimentation, which served to throw more light upon this subject, and because it represented, in a tangible form, a reaction which could not but occur in opposition to a certain school of enthusiasts in bacteriology, who began to regard all suppuration and inflammation as due to the presence of micro-organisms—a school represented by the followers of Hueter's doctrines and zealous advocates of Listerism.

¹ Virchow's Archiv. Vol. 86. "Giebt es eine Eiterung unabhängig von niederen Organismen?"

Indeed, so popular did this school threaten to become that Prof. Lister thought it necessary to utter a note of warning, and, in an address on the relation of micro-organisms to inflammation held in London before the pathological section of the International Congress of 1881, pointed out that inflammation might be due as well to nervous action, "sympathy," "irritation," etc.; and Volkmann, in his address on the changes which surgery had undergone during the last ten years, delivered at the same International Congress, argued that the belief that inflammation was due to the action of organic germs did not harmonize with the state of medical knowledge at that time—a view expressed in Cohnheim's writings.

A most important experimental contribution called forth by Uskoff's experiments was that of Orthmann.¹ Working with the assistance of Rosenbach, of Goettingen, with the strictest antiseptic precautions and with perfectly sterilized instruments, he found that he could inject even very large amounts of indifferent fluids under the skin of animals, and, as frequently as he desired, without causing an abscess or even a trace of suppuration. But as soon as he employed oil of turpentine, mercury, or other chemical irritants, he could produce phlegmons and abscesses even with minute quantities. Pus from these abscesses, when inoculated upon soils, did not lead to the development of any colonies. He did not, however, inoculate soils with the injected oil, which he had sterilized by heating in the steaming apparatus (100° C.) for half an hour.

But to these experiments of Orthmann, Councilman, of this country, now raised objections. He argued that although no micro-organisms had been found in the pus of these abscesses, they might, nevertheless, have been the cause of the suppuration; they might have subsequently entered through the aperture made in the skin by the injection-needle, the walls of the puncture-canal having been rendered necrotic by the action of the chemicals; or they might be situated in the walls of the abscess and have escaped notice, while those in the pus itself had perished. Koch had not been able to see bacteria in the pus of abscesses of rabbits with the microscope, but had found them in the abscess-walls.

¹ E. G. Orthmann, über die Ursachen der Eiterbildung. *Virch. Arch.* Vol. 90.

He therefore performed a series (sixteen) of similar experiments in Cohnheim's laboratory,¹ taking the precaution to inclose the chemical irritant (olive oil and croton oil in proportion of 5 to 1) in glass capsules or tubes, which he sterilized and introduced beneath the skin of rabbits with antiseptic precautions. The wounds healed without the least inflammatory reaction, and when this was accomplished the capsules were fractured subcutaneously. Suppuration ensued in all cases except in those where bland saline solutions were used.

Councilman therefore concluded that his experiments corroborated Orthmann's results, although he believes the theory of spontaneous suppuration being produced by micro-organisms to be in no wise affected by these experiments, since chemical irritants do not appear spontaneously in the tissues, but are either accidentally introduced or are the products of micro-organisms.

Rosenbach objected to these experiments, that although there might not be a sufficient quantity of micro-organisms adherent to the capsules to alone set up a reaction in the tissues, yet when the irritation of the chemicals was superadded, they might commence action.

But a more serious objection than this is applicable to Councilman's experiments, being the same which was made to Orthmann's, that the sterilization of the fluids injected had not been tested as to its adequacy by inoculation experiments. This precaution appears of especial importance, since Passet found that certain forms of bacteria were difficult to destroy in oily fluids.² Thus, in a series of experiments, performed in the Munich laboratory with the assistance of Prof. Frobenius, he found that spores of charbon resisted a steam temperature of 100° C. for five hours when suspended in oil, though they were destroyed in one hour when suspended in water; but that a temperature of 145° to 150° G. for one hour sufficed to kill the spores. Arguing that bacteria introduced into the tissues might have a similar resistance as charbon-spores, Passet repeated the experiments of Councilman, but exercising a complete control by means of the culture methods. He sterilized the glass capsules as he had done the charbon ones,

¹ "Zur Aetiologie der Eiterung." Virch. Arch. Vol. 92.

² Untersuchungen über die Aetiologie der eitrigen Phlegmone des Menschen. Dr. Joseph Passet. Berlin. 1885. Fischer's Bchhdlg.

and antiseptically introduced them beneath the skin of rabbits and guinea pigs. After one or two weeks he fractured them, and examined the abscesses after one or three more weeks. He found that such capsules as had been filled with pure olive oil, solution of common salt, or with small fragments of glass, did not call forth any reaction, but that oil of turpentine produced abscesses of the size of a nut in five cases out of seven, and several smaller abscesses in one case; and that croton oil mixed with olive oil (one in five) produced a similar abscess in about a week. The abscesses contained pus. Neither the fluids used, nor the pus, nor the abscess-walls contained micro-organisms when examined with the microscope or by means of culture methods.

The author therefore concludes that chemical irritants are capable of producing suppuration without the presence of micro-organisms.

Quite recently, however, Scheuerlein¹ has published similar experiments, which differ somewhat as to the results obtained. He used similar glass tubes of two sizes, one containing one drop, the other four drops of liquid (the latter about two-thirds the amount held in Passet's capsules, which contained 0.225 ccm.) which he sterilized by heating in steam of 100° C. for half an hour. These he introduced with antiseptic measures beneath the skin of rabbits. Thirty-two experiments were performed. No reaction followed the introduction of the tubes. They were fractured after one or two weeks; and in two days a tumor could be felt, increasing up to the fourth day, and decreasing after the twelfth day. Examinations made about the fourth or the eighth day revealed new formation of infiltrated connective tissue: no micro-organisms were traced by the microscope or by culture experiments, and *no suppuration ensued* in any case. The tubes were filled with oil of turpentine, croton and olive oil (1:5), ol. sinapis, caryophylli, macidis, sabinæ, cajeputi, juniperi; while others were filled with infusion of ipecacuanha (5 in 50), decoct. fruct. capsici (the same), tartar emetic, oil of cantharides and formic acid. Those of the latter group were, however, less effective in their action.

¹ Ernst Scheuerlein. Die Entstehung und Erzeugung der Eiterung durch chemisch. Reizmittel. Archiv. für klin. Chirurg. Vol. 32. II.

The conclusion arrived at was that small quantities of chemical irritants do not, in any case, excite suppuration, but only inflammation.

It appears of interest to note here that Rosenbach, so long ago as 1878,¹ found that croton oil applied to healthy bone-marrow would produce phlegmonous suppuration, which he could also produce by means of infections, but not by purely mechanical means or heat. These statements were afterwards endorsed by Kocher.

W. VAN ARSDALE.

¹ Deutsch. Zeitschr. für Chirurg. Vol. 10. P. 369.

[*To be Continued.*]